Reference: *Biol. Bull.* **180**: 301–309. (April, 1991)

# Opioid Systems and Magnetic Field Effects in the Land Snail, *Cepaea nemoralis*

MARTIN KAVALIERS AND KLAUS-PETER OSSENKOPP

*Division of Oral Biology, Faculty of Dentistry, and Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C1*

**Abstract.** Accumulating evidence shows that magnetic fields can affect a variety of opioid-mediated behavioral and physiological functions. The idea that endogenous opioids are involved in the mediation of fundamental behavioral responses in invertebrates is also gaining support. Evidence exists for opioid involvement in the mediation of nociceptive and antinociceptive ("analgesic") responses of the land snail, *Cepaea nemoralis*, and other mollusks, in a manner comparable to that in vertebrates. Exposure to various magnetic stimuli, including weak 60 Hz magnetic fields, has significant inhibitory effects on exogenous opiate-induced analgesia and endogenous opioid-mediated nociceptive responses of *Cepaea* in a manner analogous to that described for vertebrates. These effects of the magnetic stimuli are evident under both laboratory and natural conditions and include disruptions of the day-night rhythms of opioid-mediated nociception. These similar effects in *Cepaea* and rodents raise the possibility of a phylogenetic continuity in the effects of magnetic fields on basic opioid-mediated biological responses.

## Introduction

Results of field and laboratory studies show that the behavioral, cellular, and physiological functions of animals can be affected by magnetic stimuli (see reviews in Adey, 1981; Gould, 1984; Ossenkopp and Kavaliers, 1988). These diverse actions have led to speculation on the possible modes of action of magnetic fields on biological systems (Leask, 1977; Semm *et al.*, 1980; Adey, 1981; Kirschvink and Gould, 1981; Liburdy *et al.*, 1987; Liboff and McLeod, 1988; Blackman *et al.*, 1989).

Evidence has accumulated that endogenous opioid systems and opioid peptides, which are involved in the modulation of a broad range of basic functions (Akil *et al.*, 1984), can be affected by magnetic stimuli. Substantial data now indicates that time-varying magnetic fields, especially those in the extremely low frequency (ELF) range (0.10–100 Hz), affect endogenous opioid systems and the actions of exogenous opiates such as morphine (Kavaliers and Ossenkopp, 1984, 1986, 1987; Miller *et al.*, 1985; Ossenkopp and Kavaliers, 1987; Prato *et al.*, 1987). Opioid systems may, thus, be an integral part of the mechanism(s) whereby magnetic fields exert their diverse behavioral and physiological effects (Ossenkopp and Kavaliers, 1988).

Although interest has primarily focused on vertebrates, there is evidence that magnetic fields affect a variety of behavioral physiological processes in invertebrates (Gould, 1984). Recently, opioid-mediated behaviors that are sensitive to magnetic stimuli have been demonstrated in a gastropod mollusk, the land snail *Cepaea nemoralis* (Kavaliers *et al.*, 1983; Kavaliers and Ossenkopp, 1989). This paper briefly describes (i) opioid modulation of behavioral responses in mollusks and (ii) the effects of magnetic fields on opioid mediated responses and their day-night rhythms in the snail, *Cepaea*.

## Opioid Systems and Molluscs

### *General aspects*

In vertebrates, endogenous opioid peptides co-exist with diverse hormones in endocrine glands and with classical or peptide transmitters in peripheral autonomic and sensory neurones. In addition, opioid peptides are widely distributed in the central nervous system where they function as transmitters or neuromodulators. Three families of endogenous opioid peptides derived from three precursor peptides are known to date: the pro-opiomelanocortin (POMC), the pro-enkephalin, and the pro-dynorphin system. These precursors undergo differential

Received 19 July 1990; accepted 6 November 1990.

processing in various regions of the central and peripheral nervous systems, and the major cleavage products have different affinities to the three major types of opioid receptors: $\mu$, $\delta$, and $\kappa$ (Hollt, 1986).

These opioid peptides and receptors have now been identified in a variety of invertebrate taxa, strongly suggesting a phylogenetic conservation of opioid peptide structure and function (Kream *et al.*, 1980; Leung and Stefano, 1984, 1987; Scharrer *et al.*, 1988; Zisper *et al.*, 1988; Leung *et al.*, 1990; Santoro *et al.*, 1990). Results of behavioral, electrophysiological, immunological, and pharmacological studies have shown that endogenous opioid peptides and exogenous opiate agonists and antagonists have behavioral and physiological actions in invertebrates resembling those induced in mammals (Stefano, 1982, 1989; Leung and Stefano, 1987; Stefano *et al.*, 1989).

*Behavioral aspects*

*Nociception.* One of the primary roles of vertebrate opioid systems is the modulation of nociception and behavioral responses to aversive and stressful stimuli (Besson and Chaouch, 1987; Kavaliers, 1989a). In nature, animals commonly encounter aversive stimuli that can influence their survival. To effectively respond to these stimuli, organisms require: (i) a mechanism for recognizing aversive stimuli, (ii) a set of effectors that can react to the noxious stimulus, and (iii) a system for producing coordinated and directed movements and behavior in response to the stimuli. The ability of animals to recognize and physically react to aversive or noxious stimuli that can compromise their integrity is embodied in the term "nociception" (Sherrington, 1906). Nociceptors are preferentially sensitive to either a noxious stimulus or to an aversive stimulus that would become noxious if prolonged, and they code the intensity of the stimulus (Besson and Chaouch, 1987). In addition, the responses from the effectors are appropriate to the input from the receptors. Nociception can be used to provide an index of an animal's sensitivity to aversive environmental conditions and, thus, can allow for the determination of the capacity to execute adaptive behavior. Measurements of alterations in nociceptive-related responses (decreases in sensitivity-antinociception or analgesia when considered in terms of pain) are widely used to determine the behavioral and physiological status of animals following exposure to aversive, or potentially aversive, stimuli. In rodents, laboratory measures of nociception include recording of limb flexion or withdrawal (lifting a foot off an aversive, usually thermal surface); active avoidance (flinch jump, jumping, or moving from an aversive situation); and removal of the tail away from a thermal stimulus (tail-flick) (Kavaliers, 1989a).

Assays for invertebrate nociception have been developed, and nociceptive responses have been observed in invertebrates as well as vertebrates (Kavaliers, 1989a). For example, within a few seconds after *Cepaea* is placed on a surface warmed to 40°C, the snail lifts the anterior portion of its fully extended foot away from the aversive surface (Fig. 1). The behavioral end point used is the time at which the foot reaches its readily discernible maximum elevation. This "foot-lifting" behavior is not observed in snails that are exposed to temperatures normally present in their natural habitats, but becomes increasingly evident as the temperature is raised towards 40°C. This nociceptive response is comparable to the foot-lifting response exhibited by rodents when placed on a warmed surface. Similar, thermally induced nociceptive responses have also been reported for the snail, *Helix aspersa*, and the slug, *Arion alter* (Leung and Stefano, 1987; Dalton and Widdowson, 1989). A nociceptive function is also indicated for specific mechanoafferent neurons innervating the tail, parapodia, and much of the foot and body wall of the marine mollusk *Aplysia californica* (Walters and Erickson, 1986). These neurons display increasing discharge frequency in response to progressively increasing pressure, with maximal responses occurring to stimuli that could cause tissue damage (Walters, 1987). A similar graded pattern of response has been used to define the activity of classical mammalian nociceptors (Besson and Chaouch, 1987).

*Opioid mediation of nociception and antinociception.* Antinociception has been widely documented in experimental animals following exposure to diverse environmental stimuli, with both opioid and non-opioid mechanisms being implicated (Rodgers and Randall, 1988). Furthermore, it now seems clear that environmentally induced pain inhibition is an important component of an organism's defensive repertoire and hence has high adap-

**Figure 1.** Thermal 'nociceptive' response of a hydrated individual *Cepaea nemoratis* placed on a 40°C surface. The behavioral end point used is the maximum elevation of the anterior portion of the fully extended foot.

tive value (Amit and Galina, 1986). In vertebrates, the tonic activity of endogenous opioid systems can be increased by a range of environmental stimuli. In laboratory rodents, this "stress"—or environmentally induced analgesia (Amit and Galina, 1984)—can be recorded as an increased latency of a foot-lift or tail-flick response. Administration of either endogenous opioid peptides, such as enkephalin or exogenous opiate antagonists, such as the prototypic $\mu$ opiate agonist, morphine, produces similar analgesic effects. Prototypic exogenous opiate antagonists, such as naloxone or naltrexone, can reverse or attenuate these analgesic effects, and, in certain cases, can reduce nociceptive responses and induce hyperanalgesia (Martin, 1984).

Similar evidence for opioid involvement in the mediation of antinociception or analgesia and nociception is present for mollusks. Morphine, as well as the endogenous opioids $\beta$-endorphin and methionine-enkephalin, enhance, in a dose-dependent manner, the latency of the nociceptive responses of *Cepaea* and the slug, *Arion*, to a warmed surface (Dalton and Widdowson, 1989; Kavaliers *et al.*, 1983, 1985). As in mammals, maximum antinociceptive effects of morphine in *Cepaea* are seen 15–30 min after injection, with a decline to basal thermal response latencies by 60–120 min (Kavaliers *et al.*, 1983). These antinociceptive effects occur without any evident effects on the spontaneous locomotor activity or motor abilities of the animals. The antinociceptive effect of morphine is also produced by the benzomorphan levorphanol, but not by the stereoisomer dextrophan, suggesting that the receptor that interacts with these opiates has stereospecific requirements (Hirst and Kavaliers, 1987). Naloxone suppresses, and dose-dependently reverses, the analgesic effects of morphine in *Cepaea*, and reduces the response times (hyperalgesia) of particular morphological types of *Cepaea* that display elevated nociceptive responses (Kavaliers *et al.*, 1983; Kavaliers, 1989b). This further supports opioid involvement in the mediation of antinociception and nociception in *Cepaea*.

The specific $\mu$ and $\delta$ opioid agonists, (D-Ala$^2$-Me-Phe$^5$, Gly-ol)-enkephalin (DAMGO) and (D-Ala$^2$, D-Leu$^4$) enkephalin (DADLE), respectively, also have significant antinociceptive effects in *Cepaea* and *Arion*, suggesting the presence of $\mu$ and $\delta$ opioid receptors (Dalton and Widdowson, 1989; Kavaliers *et al.*, 1985). In addition, the specific $\kappa$ opiate agonist U-50,488H, has significant antinociceptive effects in *Cepaea* (Kavaliers and Ossenkopp, 1989). As in mammals, the duration of effect of U-50,488H is longer than that of morphine, and there is a low sensitivity to reversal by naloxone. Taken together with the demonstrations of $\kappa$ opioid binding sites and the immunocytochemical localization of the endogenous $\kappa$ ligand dynorphin, in invertebrates (Ford *et al.*, 1986), these antinociceptive effects raise the possibility of a $\kappa$ opioid-mediated antinociceptive system in *Cepaea*.

*Day-night rhythms of nociception.* Significant day-night rhythms are exhibited in the nociceptive responses and analgesic effects of morphine in *Cepaea*. These nocturnally and crepuscularly active snails display elevated night-time levels of nociception and morphine-induced analgesia under both field and laboratory conditions (Kavaliers *et al.*, 1990). The elevated nocturnal response latencies to a thermal stimulus are reduced by naloxone and the diel rhythm of nociception can be disrupted by pretreatment with the irreversible $\mu$ opioid receptor alkylating agent, $\beta$-funaltrexamine ($\beta$-FNA) (Kavaliers and Ossenkopp, 1991). This suggests that endogenous $\mu$ opioid systems may be involved in the generation or expression of the day-night rhythm of this measure of nociception in *Cepaea*.

*Stress-induced analgesia.* In rodents, diverse stimuli have been shown to increase endogenous opioid activity and induce integrated adaptive behavioral responses, including analgesia (Amit and Galina, 1986). Similar environmentally induced opioid activation and analgesia is also evident in mollusks and other invertebrates (Kavaliers, 1987; Maldonado and Miralto, 1987; Dalton and Widdowson, 1989; Valeggia *et al.*, 1989). Exposure to either heat, centrifugal rotation, or novel chemical stimuli has been shown to increase thermal nociceptive thresholds of *Cepaea* (Kavaliers, 1987, 1989a). The warm-stress-induced analgesia is blocked by naloxone and the $\delta$ opioid antagonist ICI 154,129, and is suppressed by a 24-h pretreatment with $\beta$-FNA (Kavaliers, 1987). Brief body (tail) pinch stress of the slugs *Arion* and *Limax* also resulted in significant increases in their response latencies (Kavaliers and Hirst, 1986; Dalton and Widdowson, 1988). The analgesic response of *Limax* was blocked by naloxone, while that of *Arion* was reduced in a dose-dependent manner by naltrexone and the $\delta$ opiate antagonist ICI 148,164. Moreover, the duration of the stress-induced analgesia in *Arion* could be prolonged by the injection of enkephalinase inhibitors (Dalton and Widdowson, 1988). This further supports the involvement of endogenous opioid peptides in the mediation of a number of forms of stress-induced analgesia in gastropod mollusks. It should be noted, however, that although these antinociceptive responses are opioid-mediated, it would be desirable to demonstrate cross-tolerance to exogenous opiate-induced analgesia, as well as to show changes in endogenous opioid peptide levels and receptor binding.

*Mechanisms.* At a biochemical and cellular level, there is evidence to suggest that the antinociceptive effects of opiates, in both *Cepaea* and rodents, are associated with alterations in calcium channel activity. Calcium channels are reported to be involved in the regulation of neuronal functions in mollusks in a manner similar, but not nec-

essarily identical, to that in vertebrates (Akaike *et al.*, 1981; Gerschenfeld *et al.*, 1986; Hammond *et al.*, 1987; Miller, 1987). In vertebrates, the activation of $\mu$ or $\delta$ opioid receptor types increases potassium channel conductance and indirectly reduces calcium channel conductance, while activation of $\kappa$ receptors causes a direct reduction in voltage dependent calcium conductance (North, 1986). In both cases, the net result is a reduction in neuronal discharge frequency and the amount of transmitter released. In both rodents and *Cepaea*, the dihydropyridine (DHP) and non-DHP calcium channel antagonists diltiazem, verapamil, and nifedipine can reduce exogenous opiate and stress-induced opioid analgesia (Kavaliers and Ossenkopp, 1987, 1989). This suggests similar roles for calcium-channel-related mechanisms in the mediation of opiate-induced analgesia in mammals and mollusks. In addition, pharmacological reductions of G protein activity by pertussis toxin pretreatment have similar inhibitory effects on morphine-induced analgesia in *Cepaea* and rodents (Yu and Kavaliers, 1991). This suggests that similar intermediary messenger systems are involved in the mediation of opiate effects in *Cepaea* and rodents. Moreover, data also indicate that opiates have similar inhibitory effects on dopamine and possibly other monoamine systems in rodents and mollusks (Stefano, 1982). These observations suggest similar modes of action and sensitivities of opioid systems in vertebrates and mollusks.

## Magnetic Fields and Opioid Systems

### *General aspects*

Research on the roles of geomagnetic information in avian and invertebrate orientation and migration has provided some of the most convincing results on the biological effects of magnetic fields (Ossenkopp and Barbeito, 1978; Gould, 1984; Wiltschko and Wiltschko, 1990). A variety of other biological effects produced by exposure to magnetic fields have also been documented in both invertebrates and vertebrates (reviews in Adey, 1981; Gould, 1984; Ossenkopp and Kavaliers, 1988). In mollusks, these effects of magnetic fields include alterations in neuronal activity and orientation behaviors (Brown and Webb, 1960; Brown *et al.*, 1960 a,b; Brown, 1971; Lohmann and Willows, 1987; Azanza, 1989; Balaban *et al.*, 1990).

As previously indicated, among the more dramatic actions of magnetic stimuli in mammals are reversible modifications in the effects of exogenous opiates and endogenous opioids. Natural geomagnetic disturbances arising from intense solar activity, earth strength, 0.5–1.5 gauss 60 Hz magnetic fields, relatively weak rotating magnetic fields, and stronger magnetic fields associated with diagnostic magnetic resonance imaging have all been shown to reduce the analgesic effects of morphine in mice (Kavaliers and Ossenkopp, 1984, 1986; Miller *et al.*, 1985; Ossenkopp *et al.*, 1983; Prato *et al.*, 1987).

Results of recent investigations with the snail *Cepaea*, have extended these inhibitory effects of magnetic stimuli on opioid systems to mollusks (Kavaliers and Ossenkopp, 1989). These, and additional findings from *in vitro* preparations (Golding *et al.*, 1985), avian orientation (Papi and Luschi, 1991), and spatial learning in rodents (Kavaliers *et al.*, 1991a), which relate the effects of magnetic fields to alterations in opioid activity, suggest that a broad range of fundamental opioid-mediated functions may be sensitive to magnetic stimuli.

### *Magnetic fields and opioid-mediated nociception in* Cepaea

*Rotating magnetic fields.* Results of investigations of the effects of exposure to a 0.5 Hz rotating magnetic field (RMF) on morphine-induced antinociception in *Cepaea* provided the first direct evidence that magnetic stimuli could affect opioid systems in an invertebrate (Kavaliers and Ossenkopp, 1989). As in rodents (Kavaliers and Ossenkopp, 1986, 1987), exposure for 15–30 min to a heterogenous time-varying magnetic field (0.15–9.0 mT or 1.5–90 gauss, produced by two rotating horseshoe magnets) of about 0.5 Hz significantly reduced day-time morphine-induced analgesia in *Cepaea* without any evident effects on the basal nociceptive responses of saline-treated control animals. The rotating magnetic fields also attenuated the analgesic effects of the $\kappa$ opiate agonist U-50,488H. In addition, and as in rodents, exposure to the rotating magnetic fields reduced stress-induced opioid analgesia in *Cepaea*. These findings show that time-varying magnetic fields can significantly alter both exogenous opiate- ($\mu$ and $\kappa$) and endogenous opioid-induced analgesia in an invertebrate. These observations also raise the possibility that exposure to magnetic stimuli may compromise the expression of adaptive opioid-mediated behavioral and physiological responses to environmental stresses. It should be noted that in control sham exposure conditions, where dummy weights rather than horseshoe magnets were used, there were no effects on opioid-mediated antinociception. In these studies there was an equivalent electric field in the sham and magnetic field exposure conditions. This minimizes the potential involvement of electric fields in the inhibition of opioid analgesia.

*60 Hz magnetic fields.* Increasing concerns about the possible health effects due to exposure to high-voltage transmission lines and electrical appliances in the home have been expressed (Ahlboom, 1988). There have been numerous reports documenting biological effects in vertebrates following exposure to 50 or 60 Hz magnetic fields, although relatively little is known about the possible effects

in invertebrates. The effects in vertebrates have included retardation in embryological development, changes in behavioral activity levels and inhibition of chemically and electrically kindled seizures. These effects are compatible with alterations in the functioning of endogenous opioid systems (review in Ossenkopp and Kavaliers, 1988). This speculation of opioid involvement is encouraged by the observation that acute (30-min) exposure to low intensity 60 Hz magnetic fields markedly reduces morphine-induced analgesia levels in mice, with a functional relationship between magnetic field intensity and the degree of inhibition of analgesia (Ossenkopp and Kavaliers, 1987).

Recently, it was observed that exposure of *Cepaea* to low intensity (1.0 gauss, rms) 60 Hz magnetic fields in a Helmholtz coil apparatus, as shown in Figure 2, also resulted in an attenuation of morphine-induced analgesia (Kavaliers *et al.*, 1990). Various durations of exposure (0.50, 2, 12, 48, or 120 h) to the 60 Hz fields reduced the levels of morphine-induced analgesia in both the light and dark periods of a 12 h light:12 h dark cycle, with the magnetic stimuli having significantly greater inhibitory effects in the dark period. The inhibitory effects of the magnetic fields were reversible. Twenty-four hours after exposure, the levels of morphine-induced analgesia were not significantly different from pre-exposure levels (Kavaliers *et al.*, 1990). These effects in *Cepaea* are consistent with the day-night rhythms in the inhibitory effects of naloxone and 60 Hz and rotating magnetic fields on morphine-induced analgesia in nocturnal rodents (Kavaliers and Ossenkopp, 1984; Ossenkopp and Kavaliers, 1987). The weak 60 Hz magnetic fields also significantly reduced the levels of the elevated naloxone-sensitive dark period nociceptive response latencies in *Cepaea*, while not affecting the lower level light period responses. Moreover, the degree of attenuation of the analgesic and nociceptive response latencies was related to the duration of exposure to the 60 Hz magnetic fields.

Determinations were also made of the effects of 60 Hz magnetic fields on opioid-mediated responses outside the laboratory under natural conditions. Exposure to 1.0 gauss 60 Hz magnetic fields under field conditions significantly attenuated morphine-induced antinociception and nociceptive responses of *Cepaea*, with the degree of attenuation being related to the duration of exposure to the magnetic fields (Tysdale *et al.*, 1991). The 60 Hz fields also disrupted the day-night rhythm of nociception, with particularly marked alterations in responses occurring during the rapidly changing light levels of the twilight periods as shown in Figure 3. These field observations suggest a possible relation between light reception or changes and magnetic field reception in *Cepaea*. A connection between magnetic field and light reception has been previously theoretically postulated (Leask, 1977) and experimentally indicated in several species of arthropods and vertebrates (Leucht, 1984, 1990; Olcese *et al.*, 1985, 1988; Phillips, 1987). However, it has not been established whether light itself is a prerequisite for reception of the magnetic field.

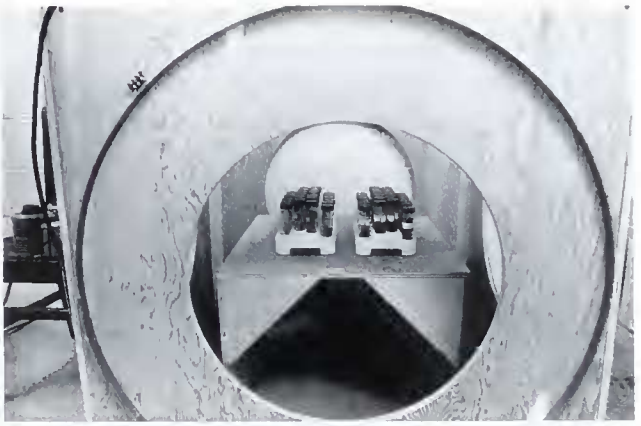

**Figure 2.** Helmholtz coil apparatus used for generation of 60 Hz magnetic fields to which the *Cepaea nemoralis* were exposed. Snails were held individually in translucent polypropylene 50 ml centrifuge tubes (10 × 2.5) containing a saturated atmosphere and natural vegetation. The tubes were placed upright on a platform (exposure volume) in the Helmholtz coil apparatus. The Helmholtz coils consisted of 100 turns of no. 24 motor enamel wire with a resistance of about 25 Ω per coil. The coils were 100 cm in diameter spaced 103 cm apart on the Z-axis and attached to the outside of a plywood frame. The coils were covered with a resin coating which immobilized the wires in the coils and prevented them from vibrating when they were carrying current. Line current (60 Hz) from standard outlets was applied to the coils and regulated with two variable autotransformers. The experimental exposure volume (30 × 30 × 330 cm) in which the snails in the tubes were placed was centered between the energized coils on the Z-axis. By altering the voltage input to the two coils, 60 Hz fields with linear polarity and field intensities up to 1.5 gauss (rms) could be generated [a field intensity of 1.0 gauss (rms) was used in the studies described in the text]. A sham field exposure condition was produced by turning off the current to the coils and placing test animals in the same exposure volume.

These observations with *Cepaea* show that exposure to weak 60 Hz magnetic fields significantly affects the diel rhythms of opioid-mediated responses in both the laboratory and under natural environmental conditions. They also show that the degree of inhibition of opioid-mediated responses is affected by both the duration and timing (day-night variations) of exposure to 60 Hz magnetic fields. These latter findings are particularly significant in view of the growing reports of associations between prolonged exposures to low intensity 50 and 60 Hz magnetic fields and the occurrence of various types of neoplasms (*e.g.*, Savitz *et al.*, 1988) and the evidence that opioid systems can modulate tumorigenesis (Zagon and McLaughlin, 1987). In this regard, it is of interest that the snails exposed to the 60 Hz magnetic fields showed, over a two-week period following exposures, increased levels of mortality relative to control sham-field exposed animals, and that night-time exposures resulted in greater mortality levels than day-time exposures (Ossenkopp *et al.*, 1990). This

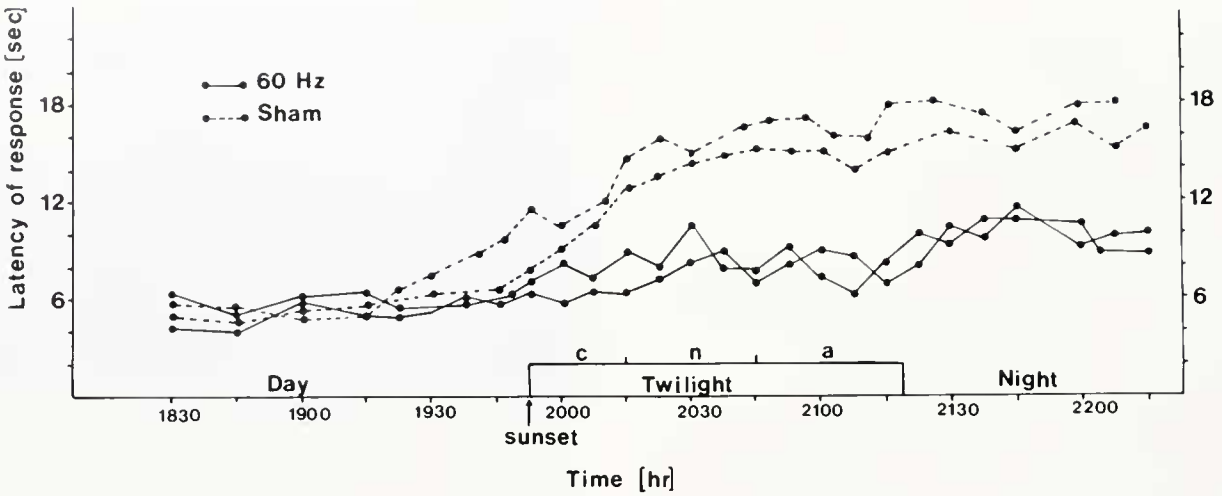


**Figure 3.** Examples of daytime, nighttime and twilight period thermal (40°C) response latencies (nociceptive responses) of *Cepaea nemoralis* held under natural summer (August) outdoor (Environmental Sciences Center, London, Ontario, 43° 4′ 30″ and 81° 18′ 30″ W) light conditions and exposed to either a 60 Hz magnetic field (1.0 gauss, rms, as described in Fig. 2) or a sham magnetic field (sham). Each point shown represents the mean nociceptive response of 18–24 snails. Different groups of snails were tested on each of the four days shown. For ease of presentation standard errors are excluded.

Exposure to the 60 Hz magnetic fields had no significant effects on the daytime (pre-sunset) nociceptive responses, but significantly ($P < 0.01$, repeated measures analysis of variance for 2200 h) reduced the nighttime response latencies as compared to the sham exposed snails and other control animals (not shown). Exposure to the magnetic fields also significantly ($P < 0.05$, for 2000 and 2100 h) attenuated the marked increases in thermal response latencies that occurred during the decreasing light levels of the twilight periods [civil (c), nautical (n) and astronomical (a) twilights; defined by the sun at −6°, −12° and −18°, respectively, from the horizon]. The greatest effects of the 60 Hz magnetic field on nociceptive responses occurred during the nautical and astronomical portions of the twilight period.

The temperatures and light intensities that the snails were exposed to ranged from 22 to 28°C and 100 to 200 $\mu w/cm^2$ (20–40 $\mu w/cm^2$ in the tubes) in the daytime, and from 14 to 22°C and 0.01 to 10 $\mu w/cm^2$ (0.001–1.0 $\mu w/cm^2$ in the tubes) in the twilight transitions and nighttime. These light (tubes) and temperature values were similar to the conditions present in the natural habitat of the snails (Kavaliers, 1989b). The background geomagnetic field had a daytime horizontal (H) intensity of 0.48 gauss, a vertical (Z) intensity of 0.24 gauss, and inclination (I) of 75. The Helmholtz coils were oriented with the x-axis oriented almost directly towards magnetic north.

is of relevance in view of the suggestions of synergistic effects between exposure to magnetic fields and environmental pollutants in the induction of neoplasms (Adey, 1987, 1990).

### *Mechanisms of action of magnetic stimuli on opioid systems*

The inhibitory effects of the magnetic stimuli observed in both the day- and night-time may arise from the increased levels of the magnetic field as compared to earth strength fields or fluctuations in field strength. Although data has been presented to suggest that both of these components can influence biological systems (Adey, 1981; Cremer-Bartels *et al.*, 1984), evidence is accumulating that the biological effects of magnetic fields are primarily due to fluctuations in field strength (Blackman *et al.*, 1985, 1989; Prato *et al.*, 1987). Furthermore, data indicate that the extent of the biological effects of weak magnetic fields are dependent on the relative intensity and orientations of both the steady state [local geomagnetic field, which varies on a day-night basis (Cremer-Bartels *et al.*, 1984) and oscillating field (Blackman *et al.*, 1985; Prato *et al.*, 1987)]. However, it should be noted that many behavioral and physiological responses show no evidence of sensitivity to fluctuating magnetic fields (Ossenkopp and Kavaliers, 1988).

Magnetic fields have been proposed to alter the properties and stability of biological membranes, their transport characteristics, and the intra- and extra-cellular distributions and flux of calcium ions (Bawin and Adey, 1976; Adey, 1981, 1989; Liboff *et al.*, 1987; Carson *et al.*, 1990). Blackman *et al.* (1985, 1989) indicated that exposure to various combinations of time-varying and local geomagnetic fields caused significant changes in the efflux of calcium ions from *in vitro* preparations of chick brain

tissue. They speculated that this effect of magnetic fields on calcium ion efflux might involve a general property of biological tissue.

There is evidence that the inhibitory effects of the magnetic fields on opioid analgesia also involve changes in the levels, flux, and distribution of calcium ions, alterations in the functioning of calcium channels, along with modifications in the coupling between opioid receptors and calcium channels. This is supported by the findings that the DHP and non-DHP calcium channel antagonists diltiazem, nifedipine, and verapamil significantly reduce, while the DHP calcium channel agonist BAY K8644, significantly enhances the inhibitory effects of rotating magnetic fields on morphine-induced analgesia in *Cepaea* and mice (Kavaliers and Ossenkopp, 1987, 1989). In addition, the inhibitory effects of rotating magnetic fields on murine morphine-induced analgesia are reduced by the calcium chelator EGTA, and potentiated by the ionophore A21387 (Kavaliers and Ossenkopp, 1986).

Magnetic stimuli could affect calcium channel activation and conductance either directly or indirectly through alterations of intermediary effector or messenger systems. The second messenger system most commonly associated with opioid receptors and changes in ion transport involves inhibition of adenyl cyclase through G proteins (North, 1986; Stryer and Bourne, 1986). Administrations of pertussis toxin, which deactivates G proteins, reduce opiate-induced analgesia in both rodents and *Cepaea* (Parenti *et al.*, 1986; Przewlocki *et al.*, 1987; Yu and Kavaliers, 1990). Whether magnetic fields affect G protein activity is not known.

Calcium-activated, phospholipid-dependent protein kinase (protein kinase C; PKC) also plays an important role in relaying transmembrane signalling in diverse calcium-dependent cellular processes (Kaczmarek, 1987). Results of studies with PKC activators and inhibitors have shown that modulation of ion channel activity is an important function of PKC (DeRiemer *et al.*, 1985; Kaczmarek, 1987; Strong *et al.*, 1987; Conn *et al.*, 1989). Relatively little is known about the relations between PKC and opioid receptor activity, although results of a recent study indicate that stimulation of PKC with phorbol esters attenuates opioid activity through a decrease in G protein activity (Louie *et al.*, 1990).

There is, however, accumulating evidence linking magnetic fields and PKC activity. Magnetic stimuli have been reported to augment the effects of phorbol esters (PKC activators) and increase PKC activity in a number of cell culture preparations (Byus *et al.*, 1987; Adey, 1987, 1990). In *Cepaea*, the isoqinoline sulfonamides H-7 and H-9, which are specific inhibitors of PKC, reduce the inhibitory effects of 60 Hz magnetic fields on morphine-induced analgesia, whereas administration of the PKC activator SC-9 augments the effects of the magnetic fields (Kavaliers *et al.*, 1991b). This suggests that the inhibitory effects of magnetic fields on opiate-induced analgesia in *Cepaea* may include increases in PKC activity. Whether this involves effects on G proteins remains to be determined.

These mechanisms of action encompass a broader range of effects than just that of the opioid systems. However, in view of the broad range and phylogentic conservation of fundamental processes in which opioid systems are involved, these findings suggest that some of the biological effects of magnetic fields may arise through alterations of opioid activity.

## Acknowledgments

We thank Susan Lipa and Donna Tysdale for their technical assistance and twilight determinations. The research described here was supported by Natural Science and Engineering and Research Council of Canada grants to M.K. and K.P.O.

## Literature Cited

**Adey, W. R. 1981.** Tissue interactions with non-ionizing electromagnetic fields. *Physiol. Rev.* **61:** 435–513.

**Adey, W. R. 1987.** Cell membranes: the electromagnetic environment and cancer promotion. *Neurochem. Res.* **13:** 671–677.

**Adey, W. R. 1990.** Joint actions of environmental nonionizing electromagnetic fields and chemical pollution in cancer promotion. *Environ. Health Perspec.* **86:** 297–305.

**Ahlbom, A. 1988.** A review of the epidemiologic literature on magnetic fields and cancer. *Scan. J. Work Enviro. Health* **14:** 337–343.

**Akaike, N., A. M. Brown, K. Nishi, and Y. Tsuda. 1981.** Actions of verapamil, diltiazem and other divalent cations on the calcium current of *Helix* neurons. *Br. J. Pharmacol.* **74:** 87–95.

**Akil, H., S. J. Watson, M. E. Lewis, H. Khachaturian, and M. J. Walker. 1984.** Endogenous opioids: biology and function. *Ann. Rev. Neurosci.* 7: 22–256.

**Amit, Z., and G. H. Galina. 1986.** Stress-induced analgesia: adaptive pain suppression. *Physiol. Rev.* **66:** 1091–1120.

**Azanza, M. J. 1989.** Steady magnetic fields mimic the effect of caffeine on neurons. *Brain Res.* **489:** 195–198.

**Balaban, P. M., N. I. Bravarenko, and A. N. Kuznetzov. 1990.** Influence of a stationary magnetic field on bioelectric properties of snail neurons. *Bioelectromagnetics* **11:** 13–25.

**Bawin, S. M., and W. R. Adey. 1976.** Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. *Proc. Nat. Acad. Sci. USA* **73:** 1999–2003.

**Besson, J.-M., and A. Chaouch. 1987.** Peripheral and spinal mechanisms of nociception. *Physiol. Rev.* **67:** 68–186.

**Blackman, C. F., S. G. Beane, R. J. Rabinoowicz, D. R. House, and W. T. Joines. 1985.** A role for the magnetic field in the radiation-induced efflux of calcium ions from brain tissue in vivo. *Bioelectromagnetics* **6:** 327–339.

**Blackman, C. F., L. S. Kinney, D. E. House, and W. T. Joines. 1989.** Multiple power-density windows and their possible origin. *Bioelectromagnetics* **10:** 115–128.

**Brown, F. A., Jr. 1971.** Some orientational influences of non-visual terrestrial electromagnetic fields. *Ann. N.Y. Acad. Sci.* **188:** 224–241.

**Brown, F. A., Jr., and H. M. Webb. 1960.** A "compass-direction" effect for snails in constant conditions and its lunar modulation. *Biol. Bull.* **119:** 65–74.

**Brown, F. A., Jr., M. F. Bennett, and H. M. Webb. 1960a.** A magnetic compass response of an organism. *Biol. Bull.* **119:** 65–74.

**Brown, F. A., Jr., W. J. Brett, M. F. Bennett, and F. H. Barnwell. 1960b.** Magnetic response of an organism and its solar relationships. *Biol. Bull.* **118:** 367–381.

**Byus, C. V., S. E. Pieper, and W. R. Adey. 1987.** The effects of low-energy 60 Hz environmental electromagnetic fields upon the growth-related enzyme ornithine decarboxylase. *Carcinogenesis* **8:** 1385–1389.

**Carson, J. J. L., F. S. Prato, D. J. Prost, L. D. Diesbourg, and S. J. Dixon. 1990.** Time-varying magnetic fields increase cytosolic free $Ca^{2+}$ in HL60 cells. *Am. J. Physiol.* **28:** C687–C692.

**Chiles, C., E. Hawrot, J. Gore, and R. Byck. 1989.** Magnetic field modulation of receptor binding. *Magnetic Resonance in Medicine* **10:** 241–245.

**Conn, J. P., J. A. Strong, and L. K. Kaczmarek. 1989.** Inhibitors of protein kinase C prevent enhancement of calcium current and action potentials in peptidergic neuron of *Aplysia*. *J. Neurosci.* **9:** 480–487.

**Cremer-Bartel, G., K. Krause, G. Mitoskas, and D. Brodersen. 1984.** Magnetic fields of the earth as additional zeitgeber for endogenous rhythms. *Naturwissenschaften* **71:** 567–574.

**Dalton, L. M., and P. S. Widdowson. 1989.** The involvement of opioid peptides in stress-induced analgesia in the slug *Arion ater*. *Peptides* **10:** 9–13.

**De Riemer, S. A., J. A. Strong, K. A. Albert, P. Greengard, and L. K. Kaczmarek. 1985.** Enhancement of calcium current in *Aplysia* neurons by phorbol ester and kinase C. *Nature* **313:** 313–316.

**Ford, R., D. M. Jackson, L. Tetrault, J. C. Torres, P. Assanh, J. Harper, M. K. Leung, and G. B. Stefano. 1986.** A behavioral role for enkephalins in regulating locomotor activity in the insect *Leucophaea maderae:* evidence for high affinity kappa-like opioid binding sites. *Comp. Biochem. Physiol.* **85C:** 61–66.

**Gerschenfeld, H. M., C. Hammond, and D. Paupardin-Tritsch. 1986.** Modulation of the calcium current of molluscan neurones by neurotransmitters. *J. Exp. Biol.* **124:** 73–91.

**Golding, G. P., L. Newboult, J. M. H. Rees, and B. R. Varlow. 1985.** The effects of 50 Hz magnetic fields on opioid peptide mediated inhibition of guinea pig ileum. *Neuropeptides* **5:** 357–358.

**Gould, J. 1984.** Magnetic field sensitivity in animals. *Ann. Rev. Physiol.* **4:** 585–598.

**Hammond, C., D. Paupardin-Tritsch, A. C. Narin, P. Greengard, and H. M. Gerschenfeld. 1987.** Cholecystokinin induces a decrease in $Ca^{++}$ current in snail neurons that appears to be mediated by protein kinase C. *Nature* **325:** 809–811.

**Hirst, M., and M. Kavaliers. 1987.** Levorphanol but not dextrophan suppresses the foot-lifting response to an aversive stimulus in the terrestrial snail (*Cepaea nemoralis*). *Neuropharmacology* **26:** 121–123.

**Hollt, V. 1986.** Opioid peptide processing and receptor selectivity. *Ann. Rev. Pharmacol. Toxicol.* **26:** 59–77.

**Kaczmarek, L. K. 1987.** The role of protein kinase C in the regulation of ion channels and neurotransmitter release. *Trends Neurosci.* **10:** 30–34.

**Kavaliers, M. 1987.** Evidence for opioid and non-opioid forms of stress-induced analgesia in the snail, *Cepaea nemoralis*. *Brain Res.* **410:** 111–115.

**Kavaliers, M. 1989a.** Evolutionary and comparative aspect of nociception. *Brain Res. Bull.* **6:** 923–931.

**Kavaliers, M. 1989b.** Polymorphism in opioid modulation of the thermal responses of the land snail *Cepaea nemoralis*. *Can. J. Zool.* **67:** 2721–2724.

**Kavaliers, M., and M. Hirst. 1986.** Naloxone-reversible stress-induced feeding and analgesia in the slug (*Limax maximus*). *Life Sci.* **38:** 203–209.

**Kavaliers, M., M. Hirst, and G. C. Teskey. 1983.** A functional role for an opiate system in snail thermal behavior. *Science* **220:** 99–101.

**Kavaliers, M., M. Hirst, and G. C. Teskey. 1985.** The effects of opioid and FMRF-amide peptides on thermal behavior in the snail. *Neuropharmacology* **24:** 621–626.

**Kavaliers, M., and K.-P. Ossenkopp. 1984.** Exposure to rotating magnetic fields alters morphine-induced behavioural responses in two strains of mice. *Neuropharmacology* **89:** 440–443.

**Kavaliers, M., and K.-P. Ossenkopp. 1986.** Magnetic field inhibition of morphine-induced analgesia and behavioral activity in mice: evidence for involvement of calcium ions. *Brain Res.* **379:** 830–838.

**Kavaliers, M., and K.-P. Ossenkopp. 1987.** Calcium channel involvement in magnetic field inhibition of morphine-induced analgesia. *Naunyn-Schmiedebergs Arch. Pharmakol.* **336:** 308–315.

**Kavaliers, M., and K.-P. Ossenkopp. 1989.** Magnetic fields inhibit opioid-mediated 'analgesic' behaviours of the terrestrial snail, *Cepaea nemoralis*. *J. Comp. Physiol. A.* **162:** 551–558.

**Kavaliers, M., and K.-P. Ossenkopp. 1991.** Magnetic fields, opioid systems and day-night rhythms of behavior. In *Electromagnetic Fields and Circadian Rhythms, Vol. I. Advances in Circadian Physiology.* M. Moore-Ede and S. Campbell, eds. Binkhauser, Boston, (in press).

**Kavaliers, M., K.-P. Ossenkopp, and S. Lipa. 1990.** Day-night rhythms in the inhibitory effects of 60 Hz magnetic fields on opiate-mediated 'analgesic' behaviors of the land snail, *Cepaea nemoralis*. *Brain Res.* **517:** 276–282.

**Kavaliers, M., L. Eckel, K.-P. Ossenkopp, and N. Yu. 1991a.** Enhanced learning of a sexually dimorphic spatial task in meadow voles following brief exposure to 60 Hz magnetic fields. *Soc. Neurosci. Abstr.* **21** (in press).

**Kavaliers, M., K.-P. Ossenkopp, and D. M. Tysdale. 1991b.** Evidence for the involvement of protein kinase C in the modulation of morphine-induced 'analgesia' and the inhibitory effects of exposure to 60Hz magnetic fields in the land snail, *Cepaea nemoralis*. *Brain Res.* (in press).

**Kirschvink, J. L., and J. L. Gould. 1981.** Biogenic magnetite as a basis for magnetic field detection in animals. *Biosystems* **13:** 181–201.

**Kream, R. M., R. S. Zukin, and G. B. Stefano. 1980.** Demonstration of two classes of opiate binding sites in the nervous tissue of the marine mollusc, *Mytilus edulis*. *J. Biol. Chem.* **225:** 9218–9224.

**Leask, M. J. M. 1977.** A physiochemical mechanism for magnetic field detection by migratory birds and homing pigeons. *Nature* **267:** 144–145.

**Leucht, T. 1984.** Responses to light under varying magnetic conditions in the honey bee, *Apis mellificia*. *J. Comp. Physiol. A.* **154:** 865–870.

**Leucht, T. 1990.** Ineractions of light and gravity reception with magnetic fields in *Xenopus laevis*. *J. Exp. Biol.* **148:** 325–334.

**Leung, M. K., and G. B. Stefano. 1984.** Isolation and identification of enkephalins in pedal ganglia of *Mytilus edulis* (Mollusc) *Proc. Nat. Acad. Sci. USA* **81:** 955–958.

**Leung, M. K., and G. B. Stefano. 1987.** Comparative neurobiology of opioids in invertebrates with special attention to senescent alterations. *Prog. Neurobiol.* **28:** 131–159.

**Leung, M. K., H. H. Boer, J. van Minnen, J. Lundy, and G. B Stefano. 1990.** Evidence for an enkephalinergic system in the nervous system of the pond snail, *Lymnaea stagnalis*. *Brain Res.* **531:** 66–71.

**Liboff, A. R., R. J. Rozek, M. L. Sherman, B. R. McLeod, and S. D. Smith. 1987.** $^{45}Ca$ cyclotron resonance in human lymphocytes. *J. Bioelect.* **6:** 13–22.

**Liboff, A. R., and B. R. McLeod. 1988.** Kinetics of channelised membrane ions in magnetic fields. *Bioelectromagnetics* **9:** 39–51.

**Liburdy, R. P., R. Tenforde, and R. L. Magin. 1986.** Magnetic-field induced drug permeability in liposome vesicles. *Radiat. Res.* **108:** 102–111.

**Lohmann, K. J., and A. O. D. Willows. 1987.** Lunar-modulated geomagnetic orientation by a marine mollusk. *Science* **235:** 231–235.

**Louie, A. K., E. S. Bass, J. Zahn, P. E. Law, and H. H. Loh. 1990.** Attenuation of opioid receptor activity by phorbol esters in neuroblastoma X Glioma NG 108-15 Hybrid cells. *J. Pharmacol. Exp. Therp.* **253:** 401–407.

**Maldonado, H., and A. Miralto. 1987.** Effects of morphine and naloxone on a defensive response of the mantis shrimp, *Squilla mantis*. *J Comp. Physiol.* **A147:** 455–459.

**Martin, W. R. 1984.** Pharmacology of opioids. *Pharmacol. Rev.* **35:** 283–323.

**Miller, R. J. 1987.** Multiple calcium channels and neuronal function. *Science* **325:** 46–52.

**Miller, D. B., C. F. Blackman, and J. S. Alie. 1985.** Behavioral responses of morphine-treated mice to ELF magnetic fields. *Bioelectromagn Soc. Meet. Abstr* **1985:** 54.

**North, R. A. 1986.** Opioid receptor types and membrane ion channels. *TINS* **9:** 114–117.

**Olcese, J., S. Reuss, and L. Vollrath. 1985.** Evidence for the involvement of the visual system in mediating magnetic field effects on pineal melatonin synthesis in the rat. *Brain Res.* **333:** 382–384.

**Olcese, J., S. Reuss, J. Stehle, S. Steinlecher, and L. Vollrath. 1988.** Response of the mammalian retina to experimental alteration of the ambient magnetic field. *Brain Res.* **448:** 325–330.

**Ossenkopp, K.-P., and R. Barbeito. 1978.** Bird orientation and the geomagnetic field: a review. *Neurosci. Biobehav. Rev* **2:** 255–270.

**Ossenkopp, K.-P., and M. Kavaliers. 1987.** Morphine-induced analgesia and exposure to 60 Hz magnetic fields: inhibition of nocturnal analgesia is a function of magnetic field intensity. *Brain Res* **418:** 356–360.

**Ossenkopp, K.-P., and M. Kavaliers. 1988.** Clinical and applied aspects of magnetic field exposure: a possible role for the endogenous opioid systems. *J Bioelect.* 7: 189–208.

**Ossenkopp, K.-P., M. Kavaliers, and M. Hirst. 1983.** Reduced morphine analgesia in mice following a geomagnetic disturbance. *Neurosci. Lett.* **40:** 321–325.

**Ossenkopp, K.-P., M. Kavaliers, and S. M. Lipa. 1990.** Increased mortality in land snails (*Cepaea nemoralis*) exposed to powerline (60-Hz) magnetic fields and effects of the light-dark cycle. *Neurosci. Lett.* **114:** 89–94.

**Papi, F., and P. Luschi. 1991.** Pigeon navigation: naloxone injection and magnetic disturbance have a similar effect on initial orientation. *Rend. Acc. Naz. Lincei* (in press).

**Parenti, M., F. Tirone, G. Giagnoni, N. Pecora, and D. Parolaro. 1986.** Pertussis toxin inhibits the antinociceptive action of morphine in the rat. *Eur. J Pharmacol.* **124:** 357–359.

**Phillips, J. B. 1987.** Specialized visual receptors respond to magnetic field alignment in the bowfly (*Calliphora vicina*). *Soc. Neurosci. Abstr.* **13:** 397.

**Prato, F. S., K.-P. Ossenkopp, and M. Kavaliers. 1987.** Nuclear magnetic resonance inhibition of morphine-induced analgesia in mice: differential effects of the static, radio-frequency and time-varying magnetic field components. *Mag. Reson. Imag.* **5:** 9–14.

**Przewlocki, R., T. Costa, J. Lang, and A. Herz. 1987.** Pertussis toxin abolishes the antinociception mediated by opioid receptors in rat spinal cord. *Eur. J. Pharmacol.* **144:** 91–93.

**Rodgers, R. J., and J. I. Randall. 1988.** Environmentally-induced analgesia: situational factors, mechanisms, and significance. Pp 107–147 in *Endorphins, Opiates and Behavioural Processes*, R. J. Rodgers and S. J. Cooper, eds. Wiley, Chichester.

**Santoro, C., L. M. Hall, and R. S. Zukin. 1990.** Characterization of two classes of opioid binding sites in *Drosophila melanogaster* head membranes. *J Neurochem.* **54:** 154–170.

**Savitz, D. A., H. Watchel, F. A. Barnes, E. M. John, and J. G. Tvrdik. 1988.** Case-control study of childhood cancer and exposure to 60-Hz magnetic fields. *Am. J Epidemiol.* **128:** 21–38.

**Scharrer, B., G. B. Stefano, and M. K. Leung. 1988.** Opioid mechanisms in insects, with special attention to *Leucophaea maderae*. *Cell Mol. Neurobiol.* **8:** 269–284.

**Semm, P., T. Schneider, and L. Vollrath. 1980.** The effects of an earth-strength magnetic field on the electrical activity of pineal cells. *Nature* **288:** 607–608.

**Sherrington, C. S. 1906.** *The Integrative Action of the Nervous System.* Yale University Press, New Haven.

**Stefano, G. B. 1982.** Comparative aspects of opioid-dopamine interaction. *Cell. Mol. Neurobiol* **2:** 167–182.

**Stefano, G. B. 1989.** Role of opioid neuropeptides in immunoregulation. *Prog. Neurobiol.* **33:** 149–153.

**Stefano, G. B., M. K. Leung, X. Zhao, and B. Scharrer. 1989.** Evidence for the involvement of opioid neuropeptides in the adherence and migration of immunocompetent invertebrate hemocytes. *Proc. Nat. Acad Sci. USA* **86:** 626–630.

**Strong, J. A., A. P. Fox, R. W. Tsien, and L. K. Kaczmarek. 1987.** Stimulation of protein kinase C recruits covert calcium channels in *Aplysia* bag cell neurons. *Nature* **325:** 714–717.

**Stryer, L., and H. R. Bourne. 1986.** G proteins: a family of signal transducers. *Ann. Rev. Cell. Biol.* **2:** 391–419.

**Tysdale, D., S. M. Lipa, K.-P. Ossenkopp, and M. Kavaliers. 1991.** Inhibitory effects of 60Hz magnetic fields on opiate-induced 'analgesia' in the land snail, *Cepaea nemoralis*, under natural conditions. *Physiol. Behav.* **49:** 53–56.

**Valeggia, C., E. Ferandez-Duque, and H. Maldonado. 1989.** Danger stimulus-induced analgesia in the crab *Chasmagnethus granulatus*. *Brain Res.* **481:** 304–308.

**Walters, E. T. 1987.** Site-specific sensitization of defensive reflexes in *Aplysia:* a simple model of long-term hyperalgesia. *J. Neurosci.* **7:** 400–407.

**Walters, E. T., and M. T. Erickson. 1986.** Directional control and the functional organization of defensive responses in *Aplysia*. *J. Comp. Physiol. A.* **159:** 339–351.

**Wiltschko, W., and R. Wiltschko. 1990.** Magnetic orientation and celestial cues in migratory orientation. *Experentia* **46:** 342–352.

**Yu, N., and M. Kavaliers. 1991.** Pertussis toxin reduces the day-night rhythm of nociception and opioid mediated antinociception in the land snail *Cepaea nemoralis*. *Peptides* (in press).

**Zagon, I. S., and P. J. McLaughlin. 1989.** Opioid antagonist modulation of murine neuroblastoma: a profile of cell proliferation and opioid peptides and receptors. *Brain Res.* **480:** 16–28.

**Zisper, B., M. R. Ruff, J. B. O'Neill, C. C. Smith, W. J. Higgins, and C. B. Pert. 1988.** The opiate receptor: a single 110 kDa recognition molecule appears to be conserved in *Tetrahymena* leech and rat. *Brain Res.* **463:** 296–304.